Effect of salt stress on soluble carbohydrates and proline content of sorghum
Effet du stress salin sur la teneur en hydrates de carbone et en proline du sorgho

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INTRODUCTION

Sorghum (Sorghum bicolor (L.) Moench) is the fourth most cultivated cereal in the world (Jones, 1985), being produced most frequently in high temperature and low rainfall areas and in soil with salinity problems (Boursier and Läuchli, 1990). It is a crop fairly tolerant to salinity (Richards, 1974), and it is more susceptible to salt stress during vegetative growth than germination (Francois et al., 1984) or maturation stage (Maas et al., 1986). Although there are significant differences between genotypes of sorghum grown in salt stress conditions (Taylor et al., 1975, Pathamanabhan and Rao, 1976).

Soil salinity inhibits the growth of cultivated plants, in a way this effect is also a function of plant species, salinity degree as well as the ionic composition of the soil solution (Roy et al., 1993).

Osmotic potential of cells growing under salt stress conditions decreases with the increase in cell organic and inorganic contents. However, high concentrations of ions, like sodium and chloride can damage the enzyme systems, so they are stored in vacuole where cell metabolism is less intensive than cytoplasm. Though the increase in soluble content of cytoplasm has been regarded as a mechanism to balance osmotic potential between cytoplasm and vacuole of cells under salt stress condition (Greenway and Munns, 1980).

The tolerance to salt stress is a complex process that involves morphological physiological and biochemical modifications. Survival and growth under saline environments are the result of adaptive processes such as ion transport and compartmentalisation, synthesis and accumulation of organic solutes, bearing to an osmotic adjustment (Fougère et al., 1991).

Adaptation of plants to osmotic stress brings about the development of a low osmotic potential similar to those in plant species from arid environments. As a single cell, few strategies can be conceivable to survive in such conditions. One of these mechanisms is the osmotic adjustment achieved by the storage of solutes within cells (Santos-Díaz and Ochoa-Alejo, 1994).
In plants under water or salt stress, proline content increases more than other amino acids, and this effect has been used as a biochemical marker to select varieties aiming to resist to such conditions (Bates, 1973). The increase in proline content is the most remarkable parameter in rice grown under salt stress conditions (Roy et al., 1992). Considering this feature, Roy et al. (1993) furnished exogen proline to rice seedlings, they found that 30 mol.m$^{-3}$ proline avoided salt stress and toxicity caused by 100 mol.m$^{-3}$ NaCl dose. Marcum and Murdoch (1994) found that *Cynodon dactylon x C. transvaalensis* accumulated 103 mol.m$^{-3}$ proline, when grown in 400 mol.m$^{-3}$ NaCl.

Increase in organic solutes can alleviate or avoid the loss of activity of several enzymes. For enzyme protection to be effective, high concentrations of organic solutes are necessary, such as 1.0 mol.m$^{-3}$. So it is very difficult to discern their role as an enzyme protector or osmotic balancer. In addition, the storage of organic solutes can still function as a source of carbon and nitrogen during stress periods (Greenway and Munns, 1980).

This work was performed aiming to evaluate the effect of salt stress on the content of the putative compatible solutes, carbohydrates and proline, of two genotypes of forage sorghum with different degrees of tolerance to salt stress.

**MATERIAL AND METHODS**

An experiment was performed in the green house of the Department of Chemistry, Rural Federal University of Pernambuco, during March 1996.

Three genotypes of sorghum (*Sorghum bicolour* (L.) Moench) from the gene bank of the Pernambuco Enterprise of Agriculture Research (IPA) were used in a pot experiment. In a previous screening (Barreto et al., 1995) 23 sorghum genotypes were evaluated for sodium chloride tolerance. Three of those were selected: IPA 322-1-3(5), IPA 02-03-01 and IPA 78-Ca84, respectively, sensitive, fairly tolerant and tolerant to salt stress.

The experimental design was entirely randomised, by a factorial combination between the sorghum genotypes and three salinity levels (0, 50 and 100 mol.m$^{-3}$ of sodium chloride) in a nutrient solution of Hoagland and Arnon (1950), with four repetitions.

Seeds were sown in plastic pots with a capacity of 300 mL, having washed sand as substrate. These were watered daily with distilled water until germination, and after with nutrient solution. Seedlings were transplanted to experimental units seven days after germination, there salt treatments commenced, which continued for two weeks.

The experimental units were polyethylene pots with a capacity of 2.5 litres. In each pot there was placed one plant, immersing its roots in the nutrient solution with or without sodium chloride, according to treatment. Nutrient solutions were renewed once a week after treatments started.

Plant leaves were collected 14 days after the salt treatment started, and they were kept in a deep freeze at -80 °C until their biochemical analysis.

**Analysis of soluble carbohydrates.** Samples of fresh leaves were weighed (0.2 g) and homogenised using 70 % ethanol. Then they were filtered and pigments were removed by the use of benzene. An aliquot of 0.2 mL of leaf extract was added to 1.0 mL of 0.2 % anthrone to react in a water bath for 10 minutes at 100 °C. Soon after, the test tube
was cooled in an ice bath and then the absorbance was read at 620 nm, according to Yemm and Willis (1954). Soluble carbohydrates were calculated by comparing sample absorbency with a standard glucose curve in a concentration range of 0 to 100 g.m$^{-3}$.

**Analysis of proline.** Samples of fresh tissue were weighed (0.25 g) and homogenised with 10 mL of 3 % sulphosalicylic acid as solvent. Then they were filtered and to the extracts were added 2.5 % ninhydrine solution and glacial acetic acid. In test tubes, the reaction mixtures were kept in a water bath at 100 ºC for 1 hour to develop the colours. Soon after removal from the water bath, the test tubes were cooled in ice bath and toluene was added to separate chromophore. The absorbance was read in a spectrophotometer at 520 nm, as indicated by Bates (1973). Proline content in fresh tissue was calculated by comparing the sample absorbencies with the standard proline curve in a concentration range of 0 to 25 g.m$^{-3}$.

**RESULTS AND DISCUSSION**

Soluble carbohydrates increased in all genotypes of sorghum due to the addition of 100 mol.m$^{-3}$ NaCl (Fig. 1A). Soluble carbohydrates increased 164 %, 105 % and 72% respectively in sensitive, fairly tolerant and tolerant genotypes (Fig. 1B). The addition of 50 mol.m$^{-3}$ NaCl to the nutrient solution did not affect sugar content in all the genotypes; however, it was slightly higher in tolerant, than in other genotypes. El-Haddad and O’leary (1994) found that soluble sugars increased in sorghum more than in atriplex, as an effect of salinity. According to those authors, soluble sugars increased between 30 % to 144 % in stressed plants of sorghum, as compared to control plants. In another study, sorghum grown in highly saline soil, showed only 28 % increase in soluble sugar content in leaves (Chavan and Karadje, 1986). *Phaseolus vulgaris* grown under stress of 100 mol.m$^{-3}$ NaCl increased the content of soluble sugars to 51 % as compared to control plants (Cachorro et al., 1993).

Sugar content of tomato leaf sap was affected differently by sodium chloride stress (Bezerra Neto, 1992). Sucrose increased and fructose decreased while glucose did not change as an effect of sodium chloride stress. As soluble carbohydrates increased roughly in the same proportion in all genotypes, two hypothesis can be submitted to explain why IPA 78Ca84 is more tolerant to salt stress than IPA 322-1-3(5) and IPA 02-03-01. One is that IPA 78Ca84 stores another compatible solute, which was not analysed in this work, or this genotype is more efficient at compartmentalising ion in a vacuole.

As shown in Fig. 1C, proline content in sorghum leaf ranged from 20 to 40 µg.g$^{-1}$ of fresh tissue. Considering this amino acid, there was only one genotype affected by salt stress. When the fairly tolerant genotype was grown in 100 mol.m$^{-3}$ NaCl, it had a 76 % increase in proline content, compared to control plants (Fig. 1D). Colmer et al. (1995) found that proline content was higher in sensitive wheat than in tolerant. In sorghum leaf, El-Haddad and O’leary (1994) reported an increase in proline content as the effect of salt stress. In leaf tissue of *Sesbania grandiflora*, Chavan and Karadje (1986) found 3.938 and 4.219 mg of proline per g of dry tissue, respectively in control plants and in plants grown in soil with 10 dS.m$^{-1}$. According to Richards (1974) it is a highly saline soil. Cachorro et al. (1993) regarded as too low, 2.75 µmol.L$^{-1}$ of proline in leaves of *Phaseolus vulgaris* grown in 100 mol.m$^{-3}$ NaCl. Indeed, this value is several times lower than the lowest results of proline content shown in this work. In *Brassica juncea* grown...
under salt stress conditions, proline content increased more than 19 times, when compared to control plants (Madan et al., 1994). In five graminea species, proline content increased from the 1st to the 11th day of stress, from 5 to 33 µmol.g⁻¹ of fresh tissue in tolerant cultivars, and from 4 to 18 µmol.g⁻¹ in sensitive cultivars (Torello and Rice, 1986). These values are quite higher than the results presented in this work, perhaps due to the plant specie feature or even to the age of leaves sampled. This hypothesis is supported by Colmer’s et al. (1995) conclusions in which they emphasised that solute accumulation in leaf blades is highly dependent on the plant age and leaf position on the plant stem. They showed that proline content is higher in older leaves than in younger ones. Comparing the role of proline in osmotic adjustment of tomato plants, Alarcon et al. (1994) concluded that this amino acid had low contribution to the increase of cell osmotic potential of plants under salt stress condition, when compared to the contribution of soluble sugars and organic acids.

Changes in proline content in several crops had been correlated with their capacity to tolerate and adapt to salinity conditions. However, the role of proline in promoting tolerance to salt stress is a polemic theme. Some researchers admit that the increase in proline content is merely a salt stress effect, rather than a cause of tolerance (Widholm, 1988; Ashraf, 1989). Nevertheless, there are other researchers that did not find any increase in proline content as result of salt stress (Naik and Joshi, 1983; Chavan and Karadje, 1986), possibly because it is a genetic feature to adapt to stress conditions. Another assumption could be the compartmentalisation of proline in the cytoplasm domain. Clarifying this question can be considered as a challenge to the microanalysis techniques available at the present.
FIGURE 1 - Carbohydrate (A) and proline contents (B) on leaf fresh matter of three genotypes of forage sorghum, grown for 14 days in nutrient solution with or without sodium chloride. Average of four replicates with respective standard deviations.
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REFERENCES


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